

Morphology and Development of a Unique Type of Pelagic Larva in the Starfish *Pteraster tessellatus* (Echinodermata: Asteroidea)

LARRY R. McEDWARD

Department of Zoology, University of Florida, Gainesville, Florida 32611

Abstract. Several unusual features characterize the morphology of the pelagic larva of the starfish *Pteraster tessellatus* and its metamorphosis into the juvenile stage: (1) morphogenesis of the supradorsal membrane during metamorphosis by fusion of 15 lobes on the aboral region of the body; (2) absence of brachiolar arms and attachment disk; (3) heterochronic acceleration of development in the water vascular system, and use of podia for attachment to the substratum at settlement; (4) radial (rather than bilateral) symmetry of the larva; and (5) congruent larval and adult axes of symmetry, and a transverse orientation of the adult rudiment within the larva. Collectively, these features demonstrate that *P. tessellatus* has a highly derived mode of development and a larva that is unique among the asteroid echinoderms. In contrast to the current interpretation of this larva as a modified pelagic brachiolaria, I suggest that the unusual larva of *Pteraster* represents an example of an apparently rare evolutionary transition in animal development: the re-evolution of pelagic larval development from benthic brooding.

Introduction

Major evolutionary transitions in the patterns of animal development are rare. Some transitions, such as the change from feeding larval development to nonfeeding larval development, involve such drastic morphological reorganization that the transition is irreversible (Strathmann, 1978). The evolution of pelagic larval development from benthic brooding has not been documented in the echinoderms. This suggests that, once lost from a clade, a pelagic dispersive stage might not be re-evolved. This is an important issue because it bears on the evolutionary

flexibility of the marine fauna and the predicted consequences of major environmental change: faunal turnover due to extinction and replacement or adaptive response through modification of development and life history. In this paper I describe the morphology and development of a unique type of pelagic larva from the starfish *Pteraster tessellatus*. The unusual features of this larva are consistent with the hypothesis that it has evolved from benthic brooding, rather than by modification of a pelagic larva (Strathmann, 1974). The significance of this finding is that *Pteraster* could provide a system for elucidating the functional and developmental changes that underlie the transition from benthic to pelagic development.

Starfish in the family Pterasteridae possess an unusual structure known as the supradorsal membrane. In most pterasterids, the supradorsal membrane is a thick (1–2 mm) layer that extends aborally from the lateral margins of the ambulacra to form a secondary covering over the body wall of the arms and disk (Fig. 1). This structure is supported above the body wall by skeletal elements (paxillae) and encloses a space, the nidamental chamber. In *Pteraster tessellatus*, the supradorsal membrane lacks skeletal ossicles, but contains muscles and mucus cells, is perforated by numerous minute spiracles, and possesses a single large osculum (Fig. 1) located in the center of the aboral surface (Ives, 1888; Fisher, 1911, pp. 355–363; Rodenhouse and Guberlet, 1946; Verrill, 1914, pp. 268–269).

The supradorsal membrane has three known functions: respiratory ventilation, defense, and reproduction. Alternating muscular contractions of the supradorsal membrane and the aboral body wall produce rhythmic expansions and contractions of the nidamental chamber. Seawater enters the chamber via ambulacral pores, flows over the respiratory papulae, and exits via the osculum (Johansen and Petersen, 1971; Nance and Braithwaite, 1981).

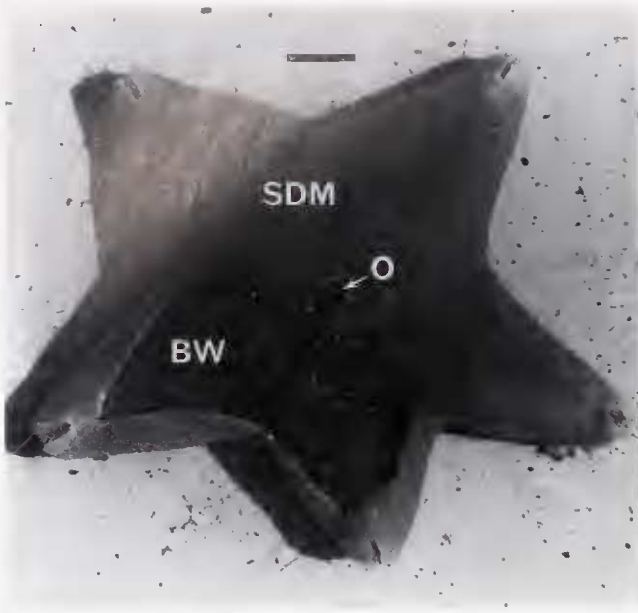


Figure 1. Adult specimen of *Pteraster tessellatus* Ives 1888. Aboral view. Scale bar = 2 cm. Supradorsal membrane (SDM) removed from two arms and part of the disk, exposing the aboral body wall (BW). Osculum (O) located center of aboral surface of supradorsal membrane.

In addition, the supradorsal membrane contains numerous mucous cells associated with the spiracles (Rodenhouse and Guberlet, 1946). Production of tremendous quantities of mucus occurs in association with expulsion of water from the nidamental chamber through the spiracles rather than the osculum. The mucus effectively deters predation by the starfish *Solaster dawsoni* and *Pycnopodia helianthoides*, possibly because of the presence of saponin-like compounds (Nance and Braithwaite, 1979). Finally, the nidamental chamber is used for brood protection (Koren and Danielssen, 1856; McClary and Mladenov, 1988). In many species of pterasterids, young are retained within the nidamental chamber of the mother throughout development to the juvenile stage (e.g., *Pteraster obscurus*, Fisher, 1911, p. 363–368; Verrill, 1914, pp. 274–277; *Pteraster stellifer*, Fisher, 1940, pp. 199–200; *Diplopteraster verrucosus*, Fisher, 1940, pp. 201–203; *Hymenaster praeoquius*, Sladen, 1889, pp. 524–525).

Benthic, brooding development has been considered the rule in pterasterids. Fisher (1940, p. 73) stated that probably all species in the genus *Pteraster* were brooders. However, Chia (1966) reported that *P. tessellatus* spawned eggs, and he provided a brief description of their development as pelagic larvae. In addition, *Pteraster militaris*, which broods embryos (Kaufmann, 1968; McClary and Mladenov, 1989, 1990), also spawns some eggs that presumably develop as pelagic larvae (McClary and Mladenov, 1988). But, beyond the observation that pelagic development occurs within the genus, very little is known

about the morphology and development of *Pteraster* larvae.

Here I report the results of a re-examination of the development of *Pteraster tessellatus* in which I either discovered or reinterpreted a number of unusual features: (1) morphogenesis of the supradorsal membrane during metamorphosis, (2) absence of brachiolar arms and attachment disk, (3) heterochronic acceleration of development in the water vascular system, (4) radial symmetry of the larva, and (5) congruent larval and adult axes of symmetry. Because of these features, the larva of *Pteraster tessellatus* is unlike that reported in any other asteroid. This description of the morphology of the pelagic larva and its development into the benthic juvenile will provide the basis for investigating the evolution of this unusual pattern of larval development (McEdward, in prep.).

Materials and Methods

Pteraster tessellatus Ives 1888 (Order Velatida; see Blake, 1987) is a subtidal, often deep-water starfish that occurs along the Pacific coast of North America from central California to the Bering Sea (Lambert, 1981, p. 88). SCUBA was used in the collection of adult starfish from depths of 5–20 m at several sites near the Bamfield Marine Station (48°49'N, 125°08'W) in Barkley Sound, Vancouver Island, British Columbia, Canada, and from depths of 15–30 m near the Friday Harbor Laboratories (48°32'N, 123°0'W) in the San Juan archipelago, Washington. *Pteraster tessellatus* is reproductive during July and August (Chia, 1966; McEdward, pers. obs.).

Females were induced to spawn by intracoelomic injection of 2–5 ml (10^{-4} M) of the hormone 1-methyl adenine. Eggs were released within 1–3 h after injection and developed without artificial insemination.

Embryos, larvae, and juveniles were cultured in plastic beakers equipped with mesh bottoms (500 μ m mesh). The beakers were suspended from a rack in an aquarium with flowing seawater (see descriptions in Hoeg, 1984; Strathmann, 1987, p. 15). The mesh bottom allowed continuous exchange of seawater between the aquarium and the culture containers. The seawater was not filtered. After the larvae hatched, \approx 250–300 healthy larvae were pipetted from each culture into clean mesh-bottom beakers for subsequent rearing.

Light microscope photographs and most of the original observations were made on living embryos, larvae, and juveniles. In some cases, larvae and juveniles were fixed and cleared to render them transparent for observation of internal features, such as the water vascular system and the skeleton. Specimens were fixed in 10% formalin in seawater (10 min), then dehydrated stepwise in ethanol (30%, 50%, 70% twice, 90%, 100%, 2 min each). Contrast was enhanced by staining the specimens with borax car-

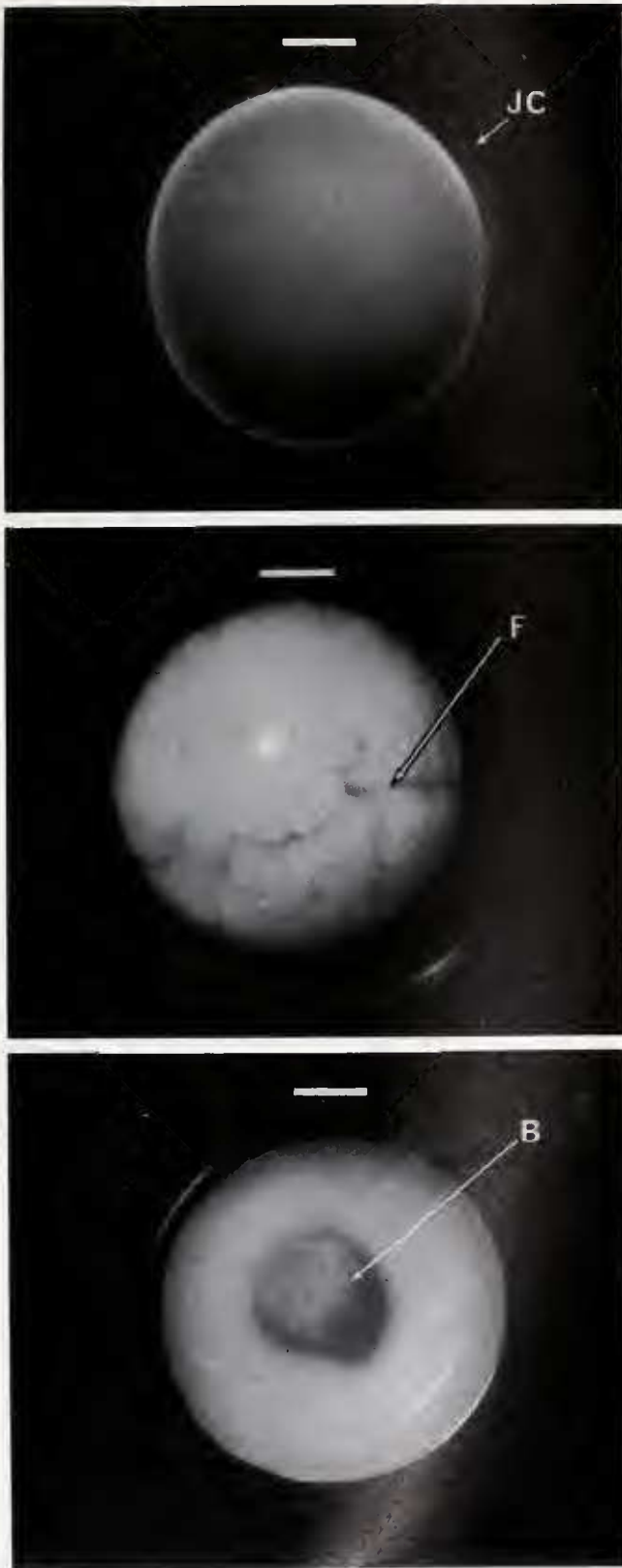


Figure 2. Light micrographs of egg and embryos of *Pteraster tessellatus*. Scale bars = 0.2 mm. (Top) Newly spawned egg. Jelly coat (JC) surrounds the egg. The vitelline layer lies between the jelly and the egg

mine (2 min) between the two changes of 70% ethanol. Immediately following dehydration, the specimens were transferred to one of three clearing agents: methyl salicylate (= oil of wintergreen), clove oil, or a mixture of benzyl alcohol and benzyl benzoate (range 1:3–3:1). Clearing was complete within 30 min to 1 h. Specimens were observed and stored in the clearing agent.

Specimens were fixed for scanning electron microscopy in cold 2% osmium tetroxide (1 h) in 0.45 μm filtered seawater, rinsed twice with distilled water, dehydrated through a graded series (30%, 50%, 70%, 15 min each) of ethanol, and stored in 70% ethanol. In preparation for drying, specimens were dehydrated stepwise to absolute ethanol (90%, 100%, 15 min each), then infiltrated with hexamethyldisilazane (HMDS, Sigma Chemical Co.) for several hours. Specimens were air-dried at room temperature (Nation, 1983) in a dust-free chamber, sputter coated with gold-palladium, and stored under desiccation.

Results

Pelagic larval development

Eggs were spawned from interradial gonopores into the nidamental chamber and carried out through the osculum of the supradorsal membrane with the exhalant flow of water. Usually between 1–10 eggs were released with each ventilation. The eggs were large, ranging in size from 1.0 to 1.4 mm in diameter (Fig. 2A). They were opaque, yolkly, and positively buoyant. Egg color varied among spawns from light yellow to dark red, but was most commonly a rich orange color. There were not obvious correlations among egg color, egg size, or the success of development. The eggs were surrounded by a thick (>100 μm) jelly coat (Fig. 2A). The jelly coat was lost prior to hatching, typically within the first 48 h of development (see Table I for chronology of development).

The cleavage pattern was variable and irregular, not the typical radial pattern characteristic of asteroids. Cleavage led to the formation of a blastula that initially had a smooth wall. With continued division of the blastomeres, the blastular wall was deeply folded to produce a wrinkled blastula (Fig. 2B). Gastrulation occurred within the vitelline envelope and involved the formation of a broad, shallow archenteron with a large blastopore at the vegetal pole (Fig. 2C). Archenteron formation was correlated with the loss of folding of the blastular wall in the vegetal hemisphere of the embryo. Subsequently, the

cell membrane but is not visible until after loss of the jelly coat. (Middle) Wrinkled blastula. Age \approx 36 h. Blastular wall with deep fold (F). Note that this is a preserved specimen and the jelly coat and vitelline layer have swollen. (Bottom) Gastrula. Vegetal view. Age = 2 days. Large blastopore (B) lies at the center of the vegetal pole.

Table I

Chronology of development in the starfish *Pteraster tessellatus* at 11–13°C

Age (days)	Developmental stage or event
0	Spawning; initiation of development
2	Wrinkled blastula; gastrulation
3	Hatching; ovoid, ciliated, swimming larva
5	Circumferential groove divides larva into two body regions
7	Podia visible within circumferential groove
8	Five marginal bulges form in posterior body region
10	Settlement; ten marginal lobes present, anterior yolky region flattened
11	Five aboral lobes begin to form
13	Aboral lobes well developed
17	Fusion of 15 lobes begins
19	Fusion of lobes complete
20	Some individuals still swimming
28	Supradorsal membrane smooth, no indication of lobes
2 (mo.)	Mouth functional
2.5 (mo.)	Distinct arms extend beyond margin of disk

blastopore was greatly reduced in size, and this occurred in association with a progressive loss of folding of the blastular wall, from the equatorial region of the vegetal hemisphere towards the animal pole. The loss of folding was completed by the time hatching from the vitelline envelope occurred.

Prior to hatching, the eggs and embryos floated at the surface. After hatching, larvae swam actively through the water column. Anterior was defined as the end that was directed forward during swimming. Hatching yielded a simple, ciliated, ovoid larva (1.1–1.2 mm length). Distinct ciliated bands for food capture were absent, and the cilia remained uniformly distributed over the surface of the larva throughout development (Fig. 3A). A larval mouth and functional gut were absent, and the blastopore closed soon after hatching; development was entirely lecithotrophic.

Within 1–2 days of hatching, a circumferential groove (Chia, 1966) formed around the larva, $\approx 1/4$ – $1/3$ of the way back from the anterior end (Fig. 3A). The groove divided the larva into two distinct body regions: an anterior region that contained mostly nutritional stores and was resorbed during development, and a posterior region that developed into the juvenile starfish. In striking contrast to most other asteroid larvae, specialized settlement structures (bra-chiolar arms and attachment disk; see Fig. 7) did not form in the anterior region at any time during development (Figs. 3, 5A).

Shortly after the formation of the circumferential groove, the posterior region of the larva shortened along the anterior-posterior axis. At the same time, five broad marginal bulges formed around the circumference of the

larva, immediately posterior to the groove (Fig. 3B). The larva continued to shorten over the next 1–2 days until it was ≈ 0.9 – 1.0 mm in length. The posterior region assumed a domed shape that was pentagonal in outline because of the broad marginal bulges. The region of the larva anterior to the groove assumed a flattened, plate-like shape (Figs. 3B, 5A).

At about the same time that the marginal bulges first became visible, podia emerged from the groove (Fig. 3B). The podia were distributed around the circumference of the larva in five clusters, each located close to the center of one of the marginal bulges. Within one week of hatching, there was an unpaired terminal podium and two pair of functional podia in each cluster. The first pair of podia

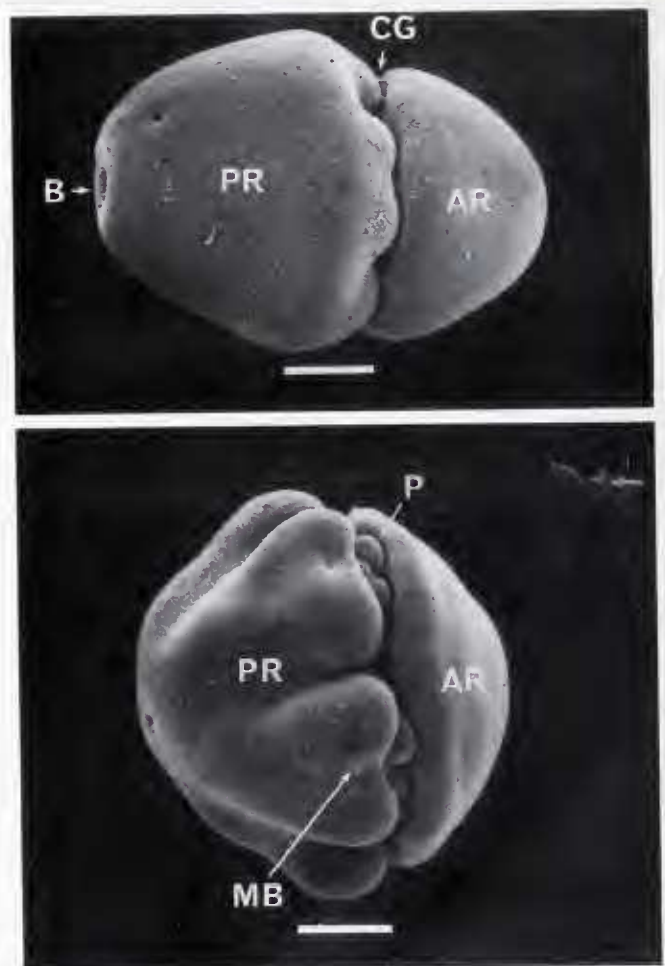


Figure 3. Scanning electron micrographs of pelagic larvae of *Pteraster tessellatus*. Scale bars = 0.2 mm. (Top) Lateral view. Age = 6 days. Circumferential groove (CG) divides the larval body into anterior (AR) and posterior (PR) regions. Note the remnant of the closing blastopore (B) at the posterior end. (Bottom) Lateral view. Age = 8 days. The terminal unpaired podium and the first pair of podia (P) of two ambulacra are visible within the circumferential groove. The bilobed marginal bulges (MB) can be seen just posterior to the groove.

was considerably longer than the second pair. The clusters became less distinct as these podia developed and additional podia formed. Eventually the podia came to be distributed in a ring around the circumference of the larva, within the groove (Fig. 5C).

Settlement and metamorphosis

Because *Pteraster* lacks purely larval structures characteristic of asteroids (e.g., ciliated bands, gut, or brachiolar arms) and does not undergo a well-defined period of metamorphosis, larval and juveniles stages cannot be rigorously defined using morphological or developmental criteria. Therefore, I have used ecological criteria: "larva" refers to the free-swimming, pelagic, dispersive stage of the life cycle and "juvenile" refers to the animal following initial settlement and assumption of the adult orientation on the substratum. In fact, the fully formed juvenile starfish was not achieved until weeks to months after settlement.

Initially larvae swam with the anterior end forward. Later, as the rudiment of the juvenile starfish developed in the posterior end, the orientation of the larval body in the water column changed and the yolky anterior end was directed upward. During settlement, the larva attached to the substratum using podia. However, attachment could only occur when the larva turned on edge because the podia were not long enough to reach around the posterior region to contact the substratum. Upon settlement, the larva placed the flattened, anterior region against the substratum, thereby assuming the definitive orientation of the adult. Settled juveniles were not fixed to the substratum and were capable of moving freely about using the podia. During the first 10 days after settlement, juveniles retained the ability to detach from the bottom and swim. Well-developed juveniles have been obtained from the plankton (F. S. Chia, pers. comm.).

Larvae that settled 2–3 days later than the majority continued to develop at the same rate as the rest, even though they remained planktonic. Likewise, juveniles that resumed swimming after initial settlement continued to develop at the same rate as those remaining on the bottom. Therefore, settlement was not coupled to a rapid, drastic metamorphosis into the juvenile form. In this respect, *Pteraster tessellatus* is similar to other asteroids with pelagic lecithotrophic larvae (e.g., *Solaster endeca*, Gemmill, 1912; *Crossaster papposus*, Gemmill, 1920) that undergo an extensive but prolonged and gradual transformation from larva to juvenile. But in contrast to most asteroid larvae, settlement of *Pteraster* did not involve fixation to the substratum nor a 90° bending (i.e., flexion, *sensu* Gemmill, 1912, p. 19) of the rudiment relative to the larval body. In all other asteroid larvae, the disk of the juvenile starfish lies in a sagittal plane in the posterior

part of the larval body, with the oral surface of the juvenile on the left side of the larva (Fig. 4). Settlement involves a bending of the larval body to bring the oral surface of the juvenile disk against the substratum. In *Pteraster*, the presumptive oral surface of the juvenile corresponds to the yolky anterior region of the larva, which corresponds to the animal pole of the embryo (site of polar body formation). The aboral surface of the juvenile disk corresponds to the posterior end of the larva and the vegetal pole (blastopore) of the embryo (Fig. 4). Since the juvenile disk lies in a transverse plane in the larval body, it does not require flexion to attain the definitive orientation with respect to the substratum following settlement.

Morphogenesis of the supradorsal membrane

Near the time of settlement, each of the five marginal bulges developed a central indentation and became strongly bilobed (Fig. 3B). Eventually they divided to yield ten distinct marginal lobes around the juvenile (Fig. 5A, B). Initially, these lobes were simple projections from the larval surface. Later, they assumed a convoluted shape and increased in size, nearly covering the lateral surface of the aboral region of the juvenile disk (Fig. 5B). Simul-

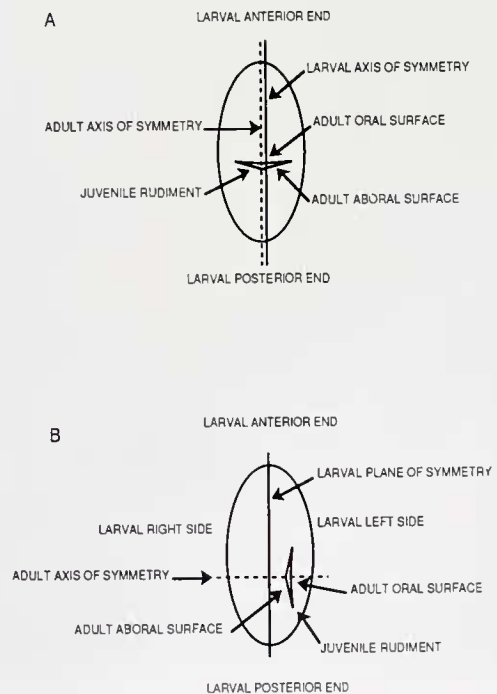


Figure 4. Diagrammatic representation of the location and orientation of surfaces, planes of section, and axes of symmetry in the larval body and rudiment of the juvenile disk of asteroids. Solid line represents the anterior-posterior axis of the larva and the dashed line represents the oral-aboral axis of the juvenile and adult. (A) Longitudinal view of the larva of *Pteraster tessellatus*. (B) Ventral view of a generalized larva representative of all other asteroids.

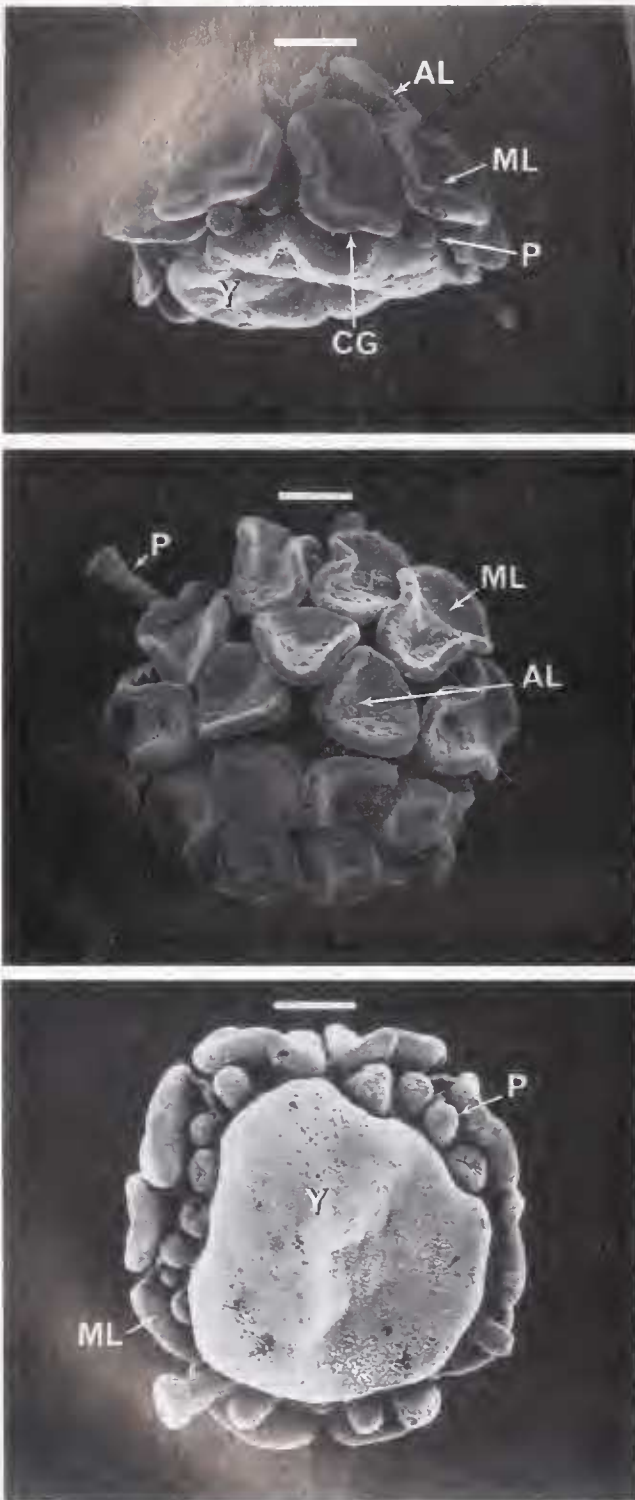


Figure 5. Scanning electron micrographs of newly settled juveniles of *Pteraster tessellatus*. Scale bars = 0.2 mm. (Top) Lateral view. Age = 16 days. Convoluted marginal lobes (ML) and aboral lobes (AL) cover the aboral surface of the juvenile. Flattened yolky plate (Y) is located below the circumferential groove (CG) and the podia (P). (Middle) Aboral view. Age = 16 days. Ten marginal (ML) and five aboral lobes (AL)

taneously with the splitting of the five marginal bulges to yield ten marginal lobes, an additional five lobes formed at the aboral pole of the juvenile (Fig. 5B). At this stage, the juvenile consisted of an oral yolk plate directed towards the substratum, a ring of podia located on the oral surface of the disk (Fig. 5C), and a developing disk with 15 convoluted lobes on the aboral surface (10 marginal and 5 aboral) (Fig. 5A).

The aboral lobes developed differently in animals from Vancouver Island compared to those from the San Juan archipelago. In animals from the San Juan Islands, the aboral lobes arose from the aboral regions of elongate marginal bulges. The marginal bulges had a triangular shape and gave rise to the three lobes (2 marginal + 1 aboral) from the vertices of the triangle. In contrast, animals from Vancouver Island produced aboral lobes independently of the marginal lobes. A central stalk developed in the center of the aboral pole of the larva and produced five rays. The tips of the rays became bulbous and developed into aboral lobes.

Subsequent development of the lobes led to the formation of the supradorsal membrane. The ten marginal lobes became organized into five sets. The lobes in a set were not derived from the same marginal bulge but instead developed from neighboring bulges. Over a period of two days, the lobes in each set enlarged and then fused along their lateral edges. The aboral lobes fused along their lateral edges and with the top (aboral) edge of the marginal lobes. Later, fusion occurred among all of the lobes to produce a complete secondary covering over the aboral surface of the juvenile starfish (Fig. 6A). At the margin of the disk, the oral edges of the marginal lobes extended beyond the disk to form a skirt around the body. Fusion along the lateral edges of the marginal lobes from adjacent sets (*i.e.*, between lobes derived from the same original marginal bulge) was restricted to the aboral regions, leaving a cleft extending between them from the oral edge of the marginal skirt (Fig. 6A). This cleft was located above the ambulacral cluster of podia and marked the site where the juvenile arms would form later in development. The osculum was formed by the lack of fusion along the central edges of the five aboral lobes, which left a central opening in the supradorsal membrane (Fig. 6B). Within ten days of the start of lobe fusion, the supradorsal membrane was completely smooth, without any visible indication that it had formed from 15 separate elements. Ventilation of the nidamental chamber by muscular pumping of the supra-

cover the aboral surface. Podia (P) can be seen between some of the marginal lobes and extending beyond the edge of the disk. (Bottom) Oral view. Age = 12 days. The ten marginal lobes (ML) can be seen around the edge of the disk of the juvenile. Podia (P) are arranged in a ring around the yolky plate (Y).

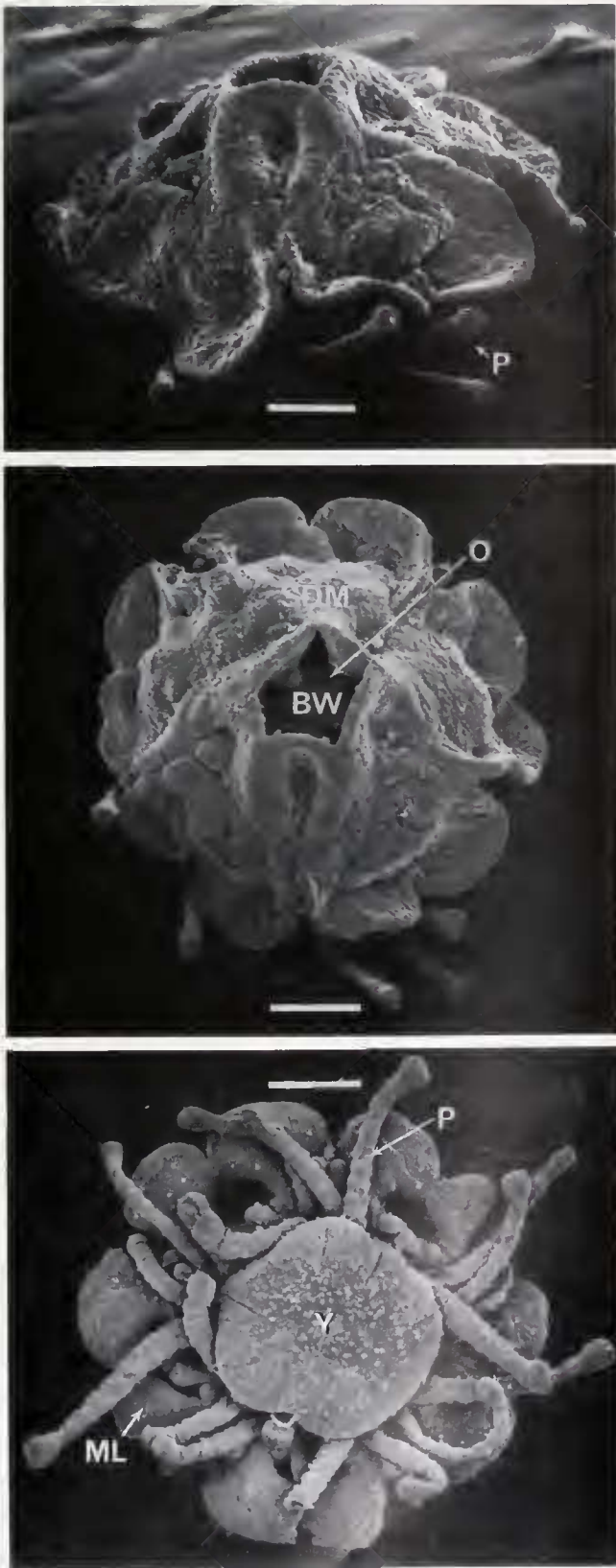


Figure 6. Scanning electron micrographs of juveniles of *Pteraster tessellatus*. Scale bars = 0.2 mm. (Top) Lateral view. Age = 27 days.

dorsal membrane (as occurs in adults) was not observed in the juveniles, even six months after settlement (diameter ≈ 2.0 – 2.1 mm). Ciliary activity was detected on the external surface of the juvenile, including the supradorsal membrane, but internal currents that might ventilate the nidamental chamber could not be demonstrated with dye streams. Mucus production was not obvious in juveniles up to ≈ 9 months after settlement (diameter ≈ 2.1 – 2.3 mm).

Chronology of larval development

Larvae and juveniles were raised at ambient seawater temperatures that ranged between 11–13°C. The schedule of events during development of the larval and early juvenile stages is listed in Table I. Development was remarkably synchronous throughout all of the cultures and among the larvae from different parents. However, the age at settlement varied greatly. The majority (>80–95%) of the larvae settled initially between days 10–12, but some continued to swim (or resumed swimming) until the third week. As indicated above, the schedule of development was not influenced by the age at which settlement occurred because the development of juvenile structures proceeded normally in swimming larvae. Morphogenesis of the supradorsal membrane was largely completed within 7–10 days following settlement. At the age of 1 month, the juveniles measured ≈ 1.5 – 1.8 mm in diameter, and the oral surface was still covered by a remnant of the yolk from the anterior region of the larva (Fig. 6C). The juvenile mouth had not yet formed and would not appear until the end of the second month. There were no indications of the juvenile arms. This resulted in a circular arrangement of the podia (3–4 pair per ambulacral cluster) around the oral surface of the disk. A radial arrangement of podia is evident in most starfish larvae at the time of settlement when the first two pair of podia are developing on the juvenile arms (Fig. 7). Distinct arms were not present in most *Pteraster* juveniles until the third month.

Discussion

Pattern of development in Pteraster tessellatus

The present study confirms the observation by Chia (1966) that *Pteraster tessellatus* has free-swimming larvae

Aboral surface covered by the supradorsal membrane. Podia (P) extend from under the oral side of the margin of the supradorsal membrane. (Middle) Aboral view. Age = 27 days. Aboral surface covered by a complete supradorsal membrane (SDM) formed from the fusion of the nidamental chamber through the large central osculum (O). (Bottom) Oral view. Age = 28 days. Remnant of the yolk plate (Y) lies in the center of the disk. The mouth has not formed yet. The free edges of the marginal lobes (ML) define the outer limit of the disk. Numerous podia (P) lie in a circle around the yolk plate. Note that the juvenile arms have not formed yet.

and presumably pelagic development. Eggs were forcefully expelled from the nidamental chamber through the osculum by the ventilatory flow. Eggs and embryos were positively buoyant, as were larvae until close to the time of settlement (8–10 days). Larvae were uniformly ciliated and swam actively near the surface of the water, in the laboratory. Later, larvae swam near the bottom and attached to solid substrata, resulting in settlement from plankton to benthos. *Pteraster* larvae and juveniles have been obtained from the plankton in the San Juan archipelago (F. S. Chia, pers. comm.; R. Emllet, pers. comm.; R. Strathmann, pers. comm.).

My observations also confirm the lack of brooding in this species. Chia (1966) did not find any brooded young in the 12 animals that he dissected. I have dissected (*i.e.*, opened or removed the supradorsal membrane) of >50 starfish without finding any evidence of brooding. Likewise, brooding has not been reported for this species by any of the authors investigating other (nonreproductive) aspects of *Pteraster* biology (Fisher, 1911, pp. 355–363; Verrill, 1914, pp. 268–269; Rodenhouse and Guberlet, 1946; Mauzey *et al.*, 1968; Johansen and Petersen, 1971; Nance and Braithwaite, 1979, 1981).

Comparisons with previous descriptions of pterasterid development

The only previous descriptions of morphological development in pterasterids are a preliminary study of development in *Pteraster tessellatus* by Chia (1966) and a brief report on brooding in *P. militaris* (Kaufman, 1968). My observations on *P. tessellatus* confirm many of Chia's descriptions: egg size, egg color, jelly coat, wrinkled blastula, large blastopore that later closes, ovoid early larva, circumferential groove, anterior (= animal) and posterior (= vegetal) body regions, resorption of the anterior region, rudiment development in the posterior region, and the chronology of early development (days 1–6).

However, there are also a number of substantial differences. Chia reported that the arms of the juvenile starfish formed early in development (p. 508): "As soon as the two parts [of the larval body] were clearly distinguishable, five primordial arms of the young seastar appeared simultaneously in the vegetal part of the larval body and the first pair of tube feet appeared on each arm (fig. 5)". According to his chronological description, the arms were formed by day 10 and the podia by day 13. I did not observe arms even after one month (Fig. 6C). Probably the initial five marginal bulges were misinterpreted as the primordial juvenile arms. My observations show that the marginal bulges are well developed by day 9, they are arranged radially around the rudiment of the juvenile disk, and they have clusters of podia associated with them (Fig. 3B).

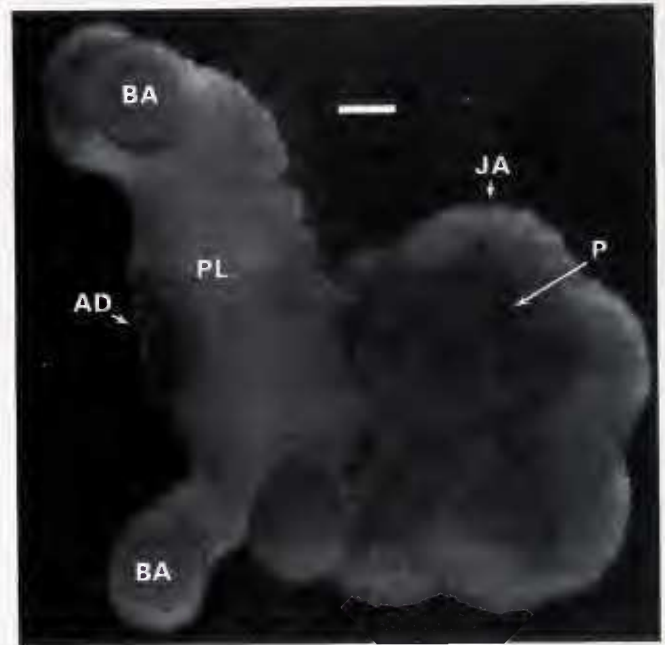


Figure 7. Light micrograph of a late-stage brachiolaria larva of *Henricia sp. (leviuscula?)*. Scale bar = 0.2 mm. Lateral view from the left side of the larva (= oral side of juvenile). Age = 28 days. Brachiolar arms (BA) and attachment disk (AD) located at anterior end on the preoral lobe (PL). Each juvenile arm (JA) has two pairs of developing podia (P) and a terminal unpaired podium on the oral surface. The plane of larval bilateral symmetry is parallel to the plane of the photograph.

I observed that most of the larvae settled between days 10–12. Chia reported that settlement occurred on the 25th day. I attribute this difference to the conditions under which the larvae were raised. A microbial and diatomaceous film developed on the mesh bottoms of my culture containers because they were suspended in unfiltered seawater and were not cleaned except when the larvae hatched (day 3). Larvae settled readily on the filmed mesh, starting on day 10. Both texture and film seem important components of attractive surfaces for larval settlement in *Pteraster*. Larvae never settled on the smooth, but filmed, sides of the culture containers. Furthermore, larvae from the same spawn that were held in closed culture containers, which were cleaned periodically, did not settle until at least one week later than larvae in mesh-bottom containers. I have observed this difference in settlement with the lecithotrophic larvae of other asteroid species that I have cultured in the laboratory: *Solaster stimpsoni*, *S. dawsoni*, *S. endeca*, *Crossaster papposus*, *Henricia sp. (leviuscula?)*. Chia cultured larvae of *Pteraster tessellatus* in a small glass aquarium (pers. comm.) that probably lacked an attractive surface (film or texture) for settlement.

Differences in the reported time of appearance of the podia (2nd pair: day 25, Chia; day 9 present study) or the number of podia present at a given stage can be explained

by the difficulty of observing the podia; they develop deep within the circumferential groove and are nearly hidden, unless they are extended for exploration of the substratum (Figs. 3B, 5A).

Other differences are less readily explained by culture conditions or interpretation of structures. Chia reported that the yolk mass was absorbed by day 25 and that the mouth of the juvenile was open on day 30. A substantial portion of the yolk plate was present on larvae in my cultures on day 28, and the mouth did not open until a month later. More puzzling is that Chia's description did not mention the marginal or aboral lobes, nor the formation of the supradorsal membrane. These were among the most outstanding features of the development of *Pteraster* larvae in my cultures (Fig. 5A, B). He does describe the aboral epidermis as wrinkled and lacking spines or ossicles. This suggests that the aboral surface was covered by the supradorsal membrane because, in *Pteraster tessellatus*, the supradorsal membrane lacks ossicles. I observed well-developed paxillae, by day 15, in juveniles that had been cleared to make the supradorsal membrane transparent. They should have been visible externally if the supradorsal membrane had not yet formed in Chia's cultures. Further, no mention was made by Chia of the lack of brachiolar structures, the unusual symmetry of the larva, or the orientation of the juvenile rudiment within the larval body.

The striking differences between these two studies raise interesting questions about development within the family Pterasteridae. Development in *Pteraster tessellatus* may be extremely variable, because I observed marked differences in the mode of aboral lobe formation in larvae from different geographic regions. Alternatively, could we have examined two different species? The systematics of the genus *Pteraster* in the northeastern Pacific Ocean has not been examined since the classic works of Fisher (1911) and Verrill (1914), and the family Pterasteridae is not well known (M. Downey, pers. comm.). Clearly, the explanation of the reported differences in development of *P. tessellatus* must await further examination of geographic variation in development and analysis of specific and subspecific systematics. Finally, Chia may have described larvae undergoing abnormal development. He reported that only 2% of the eggs in his cultures developed (Chia, 1966, p. 507), and the larvae in his Figures 3 and 5 do not resemble the larvae that I observed. However, some of these larvae successfully completed development into apparently healthy juvenile starfish.

The development of only one other pterasterid has been described. *Pteraster militaris* broods its young within the nidamental chamber (Koren and Danielssen, 1856). Recently it was shown that only a fraction of the eggs are retained and brooded, the rest are spawned through the osculum into the water column (McClary and Mladenov,

1988). Presumably the spawned eggs develop as pelagic larvae, but their development has not been described.

Development of brooded embryos of *P. militaris* was briefly described by Kaufman (1968). The embryos were divided by a constriction (= circumferential groove) into two hemispherical regions: an oral region consisting of yolk that was eventually resorbed, and an aboral region consisting of the rudiment of the body of the starfish. Five radial (= marginal) bulges developed around the aboral region. Subsequently, ten round tubercles (= marginal lobes) developed from the five radial bulges. Podia developed between the tubercles. Later, five "arms" developed at the aboral end. Kaufman misinterpreted these aboral "arms" as the arms of the juvenile starfish (see his Fig. 2). They are probably equivalent to the five aboral lobes of *Pteraster tessellatus*, and are therefore destined to be incorporated into the supradorsal membrane. Surprisingly, Kaufman did not mention the formation of the supradorsal membrane. He stated that, when the juvenile starfish was 1.5–2.0 mm in diameter (7–10 days after formation of the constriction), the madreporite opened near the center of the aboral surface. I interpret this to be the osculum of the supradorsal membrane. The young emerged from the nidamental chamber of the mother through transient slits in the supradorsal membrane.

P. militaris clearly broods at least some of its young. However, if the embryos were artificially removed from the brood chamber, they could swim via the uniform ciliation and could develop normally (Kaufman, 1968). The free-swimming embryos became benthic at the same stage that larvae of *P. tessellatus* settled. The similarity between these two species suggests that, at least in some cases, there are not major morphological differences between benthic brooding and pelagic larval development within the Pterasteridae.

Unusual features of development in Pteraster tessellatus

The larval development of *Pteraster tessellatus* is very different from what has been observed in other asteroids, including velatids [e.g., *Solaster endeca* (Gemmill, 1912), *Crossaster papossus* (Gemmill, 1920), *Solaster stimpsoni* and *S. dawsoni* (McEdward, unpubl. obs.)], and other orders (see reviews by Hyman, 1955; Fell, 1967; Oguro *et al.*, 1988).

Morphogenesis of the supradorsal membrane. The most striking feature of the development of *Pteraster tessellatus* is the formation of 15 elaborate lobes on the external surface of the posterior region of the larva (Fig. 5A, B). This feature alone makes the pelagic larva of *P. tessellatus* unique among asteroids. The lobes fuse during the transformation of the larva into the juvenile to produce the supradorsal membrane (Fig. 6A, B). It is not surprising that a pterasterid larva has an unusual morphology, given

that metamorphosis involves the formation of a highly specialized structure found only in that family. Why the supradorsal membrane forms so early in development, before other juvenile features such as the arms and the mouth, is not known.

Lack of brachiolar structures. All asteroids, except paxillosids, develop specialized larval attachment structures consisting of the brachiolar arms and disk (Fig. 7) (Oguro *et al.*, 1988). The brachiolar arms are for temporary attachment to the bottom during exploration of the substratum, and the attachment disk is generally used to cement the settled larva to the substratum during metamorphosis (Barker, 1978). *P. tessellatus*, which is a velatid, not a paxillosid, has a larva that does not form brachiolar structures during development (Fig. 3A, B). Instead, podia are used for attachment during settlement.

Accelerated development of the water vascular system. Functional podia developed very early in *Pteraster* (day 7) compared to other species from the same geographic region with pelagic lecithotrophic larval development (*e.g.*, *Solaster stimpsoni* 25–30 days, *S. dawsoni* \approx 30 days, *Crossaster papposus* \approx 50 days, *Henricia* sp.: 25–35 days; McEdward, unpubl. obs.). Because *Pteraster* reproduces in the summer and all of these other species develop in the early spring, the rapid development of *Pteraster* might be partly the result of higher seawater temperature (11–13°C in summer vs. 7–9°C in spring). However, the podia in *Pteraster* not only develop at a younger age, they are also accelerated relative to other juvenile structures such as the arms. In other asteroids with pelagic lecithotrophic larval development, there are generally two pair of podia and an unpaired terminal podium developing in the ambulacrum of each juvenile arm at settlement (Fig. 7). These podia do not become functional until days or weeks later. In contrast, there were several pair of functional podia in *Pteraster* long before the formation of the juvenile arms (Table I; Fig. 6C). Heterochronic acceleration of podial development in *P. tessellatus* provides a means of attaching to solid substrata at settlement in the absence of the typical larval attachment structures, the brachiolar apparatus.

Symmetry of the larva. The pelagic larva of *Pteraster* is radially symmetrical, based on external morphology (Fig. 3A, B) and the arrangement of internal structures (*e.g.*, coelomic cavities and water vascular system, Janies and McEdward, in prep.). All other asteroid larvae that have been described are bilaterally symmetrical (Fig. 7). *P. tessellatus* and *P. militaris* are the only asteroids known to lack a bilateral stage in the life cycle.

Larval and juvenile axes. The disk of the juvenile starfish lies in a transverse plane within the larval body of *Pteraster* (Figs. 3B, 5A) so that the anterior-posterior axis of the larva is parallel to the oral-aboral axis of the juvenile (Fig. 4). The anterior end of the larva corresponds to the oral surface of the juvenile and the posterior end of the

larva becomes the aboral surface. The orientation of the juvenile disk within the larva is very different in all other starfish. Typically, the disk lies in a sagittal plane such that the larval and juvenile axes are perpendicular and the oral surface of the juvenile develops on the left side of the larva and the aboral surface develops on the right (Figs. 4, 7) (see review by Hyman, 1955).

Collectively, these features of morphology and development distinguish the pelagic larva of *P. tessellatus* from all other asteroid larvae. The distinctiveness of the *Pteraster* larva raises the question of its evolutionary origin. It has been considered to be a lecithotrophic, modified brachiolaria larva (Chia, 1966; Fell, 1967; Oguro *et al.*, 1988), presumably derived from an ancestor with pelagic development. This interpretation is unlikely because it requires modification of fundamental and highly conservative features of larval morphology and metamorphosis while those features were functional in the life cycle. As an alternative explanation, the unusual larva of *P. tessellatus* might have evolved from an ancestor that brooded its young (Strathmann, 1974). I suggest that most of the unusual features (namely 1, 2, 4, and 5 from the list above) can be interpreted as evidence of a highly derived mode of development associated with the evolution of the specialized form of brooding in the pterasterids. The unique structural modifications of adult pterasterids, which provide a brood chamber for the young, attest to the extreme specializations towards brooding that have evolved in this group. The evolution of this type of brooding probably occurred during the radiation of the pterasterids in the deep sea. Brooding development throughout much of the evolutionary history of the pterasterids could have led to the reduction and eventual loss of larval characteristics in the offspring. For instance, the brachiolar structures were probably lost in association with an entirely benthic life cycle, where settlement structures are not needed. I postulate that the degree of reduction of larval features has been more extensive in pterasterids than in other asteroids. This does not require different selective forces acting on pterasterid development, but rather could simply be a function of differences in the evolutionary duration of the brooding pattern of development in different taxa. The relative stability of environmental conditions in the deep sea over geological time scales could have resulted in greater species longevity for pterasterid brooders compared to brooding asteroids in other taxa in shallow water. The result of extreme reduction and simplification would be highly direct development from the embryonic stage to the juvenile stage (McEdward, 1989, and in prep.; Janies and McEdward, 1991). This would explain nearly all of the unusual features in the development of *Pteraster tessellatus*. Subsequent re-evolution of pelagic development, probably in a shallow-water ancestor of *P. tessellatus*, resulted in a larval form that was distinctly different from

that of all other asteroids. Accelerated podial development was probably a key event in the re-evolution of pelagic development; it resulted in functional attachment structures that replaced the lost brachiolar complex and facilitated settlement to the benthos (McEdward, in prep.).

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Literature Cited

- Barker, M. F. 1978. Structure of the organs of attachment of brachiolaria larvae of *Stichaster australis* (Verrill) and *Coscinasterias calamaria* (Gray) (Echinodermata: Asteroidea). *J. Exp. Mar. Biol. Ecol.* **33**: 1–36.
- Blake, D. B. 1987. A classification and phylogeny of post-Paleozoic sea stars (Asteroidea: Echinodermata). *J. Nat. Hist.* **21**: 481–528.
- Chia, F.-S. 1966. Development of a deep-sea cushion star, *Pteraster tessellatus*. *Proc. Cal. Acad. Sci.* **34**: 505–510.
- Fell, H. B. 1967. Echinoderm ontogeny. Pp. S60–S85 in *Treatise on Invertebrate Paleontology, Part 5, Echinodermata*, R. C. Moore, ed. Geological Society of America and Kansas Univ. Press, Lawrence, Kansas.
- Fisher, W. K. 1911. Asteroidea of the North Pacific and adjacent waters. Part 1, Phanerozoonia and Spinulosa. *Smithsonian Inst. Bull. U. S. Nat. Mus.* **76**: 419 pp.
- Fisher, W. K. 1940. Asteroidea. *Discovery Rep.* **20**: 69–306.
- Gemmill, J. F. 1912. The development of the starfish *Solaster endeca* Forbes. *Trans. Zool. Soc. Lond.* **20**: 1–71.
- Gemmill, J. F. 1920. The development of the starfish *Crossaster papposus*, Muller and Troschel. *Q. J. Microsc. Sci.* **64**: 155–190.
- Hoeg, J. T. 1984. A culture system for rearing marine invertebrate larvae and its application to larvae of rhizocephalan barnacles. *J. Exp. Mar. Biol. Ecol.* **84**: 167–172.
- Hyman, L. H. 1955. *The Invertebrates, Vol. 4, Echinodermata*. McGraw-Hill, New York. 763 pp.
- Ives, J. E. 1888. On two new species of starfishes. *Proc. Acad. Nat. Sci. Phil.* **40**: 421–424.
- Janies, D. A., and L. R. McEdward. 1991. Evolutionary significance of a derived mode of coelom formation in the larva of the starfish, *Pteraster tessellatus*. *Am. Zool.* **31**: 105A.
- Johansen, K., and J. A. Petersen. 1971. Gas exchange and active ventilation in a starfish, *Pteraster tessellatus*. *Z. vergl. Physiol.* **71**: 365–381.
- Kaufman, Z. S. 1968. The postembryonic period of development of some White Sea starfish. *Sov. J. Mar. Biol.* **18**: 507–510.
- Koren, J., and D. C. Danielssen. 1856. Observations sur le développement des astéries. *Fauna Littoralis Norvegiae* **2**: 55–59.
- Lambert, P. 1981. *The Sea Stars of British Columbia*. British Columbia Prov. Mus., Victoria. 153 pp.
- Mauzey, K. P., C. Birkeland, and P. K. Dayton. 1968. Feeding behavior of asteroids and escape responses of their prey in the Puget Sound region. *Ecology* **49**: 603–619.
- McClary, D. J., and P. V. Mladenov. 1988. Brood and broadcast: a novel mode of reproduction in the sea star *Pteraster militaris*. Pp. 163–168 in *Echinoderm Biology*, R. D. Burke, P. V. Mladenov, P. Lambert, and R. L. Parsley, eds. Balkema, Rotterdam.
- McClary, D. J., and P. V. Mladenov. 1989. Reproductive pattern in the brooding and broadcasting sea star *Pteraster militaris*. *Mar. Biol.* **103**: 531–540.
- McClary, D. J., and P. V. Mladenov. 1990. Brooding biology of the sea star *Pteraster militaris* (O. F. Muller): energetic and histological evidence for nutrient translocation to brooded juveniles. *J. Exp. Mar. Biol. Ecol.* **142**: 183–199.
- McEdward, L. R. 1989. Development and evolution of a novel type of starfish larva. *Am. Zool.* **29**: 114A.
- Nance, J. M., and L. F. Braithwaite. 1979. The function of mucous secretions in the cushion star *Pteraster tessellatus* Ives. *J. Exp. Mar. Biol. Ecol.* **40**: 259–266.
- Nance, J. M., and L. F. Braithwaite. 1981. Respiratory water flow and production of mucus in the cushion star, *Pteraster tessellatus* Ives (Echinodermata: Asteroidea). *J. Exp. Mar. Biol. Ecol.* **50**: 21–31.
- Nation, J. L. 1983. A new method using hexamethyldisilazane for preparation of soft insect tissue for scanning electron microscopy. *Stain Technol.* **58**: 347–351.
- Oguro, C., M. Komatsu, and Y. T. Kano. 1988. Significance of the nonbrachiolarian type of development in sea-stars. Pp. 241–246 in *Echinoderm Biology*, R. D. Burke, P. V. Mladenov, P. Lambert, and R. L. Parsley, eds. Balkema, Rotterdam.
- Rodenhouse, I. Z., and J. E. Guberlet. 1946. The morphology and behavior of the cushion star *Pteraster tessellatus* Ives. *Univ. of Washington. Publ. Biol.* **12**: 21–48.
- Sladen, W. P. 1889. Report on the Asteroidea collected by the H.M.S. Challenger. *Challenger Rep., Zool.* **30**: 888 pp.
- Strathmann, M. F. 1987. *Reproduction and Development of Marine Invertebrates of the Northern Pacific Coast*. Univ. Washington Press, Seattle, Washington. 670 pp.
- Strathmann, R. R. 1974. Introduction to function and adaptation in echinoderm larvae. *Thal. Jugoslav.* **10**: 321–339.
- Strathmann, R. R. 1978. The evolution and loss of feeding larval stages of marine invertebrates. *Evolution* **32**: 894–906.
- Verrill, A. E. 1914. Monograph of the shallow-water starfishes of the North Pacific coast from the Arctic Ocean to California. *Smithsonian Inst., Harriman Alaska Series* **14**: 408 pp.